

Application No.: 10/506,693
Attorney Docket No.: 47675-86
First Applicant's Name: Kurt Berlin
Application Filing Date: April 21, 2005
Office Action Dated: January 3, 2008
Date of Response: July 3, 2008
Examiner: Katherine D. Salmon

IN THE CLAIMS:

Applicants, pursuant to 37 C.F.R. § 1.121, submit the following amendments to the claims:

1. (Currently amended) A method for detecting the presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cellular-proliferative disease in a tissue, cell type or organ of a human~~, comprising:

obtaining a bodily fluid sample from a test human;
determining an amount or presence of free floating DNA that originates from a particular tissue, cell type or organ in the sample comprising analysing for a DNA methylation pattern that is characteristic for the particular ~~tissue, cell type or organ~~; and

determining the presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cell-proliferative disease~~ based on comparing the amount or presence of free floating DNA that originates from the particular ~~tissue, cell type or organ of the test human~~, with that of a normal control value.

2. (Currently amended) A method for detecting the presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cell-proliferative disease in a tissue, cell type or organ of a human individual~~, comprising:

obtaining a bodily fluid sample from a test human ~~an individual~~;
determining an amount of total free floating DNA in the sample;
determining an amount of free floating DNA that originates from a particular tissue, cell type or organ in the sample comprising analysing for a DNA methylation pattern that is characteristic for the ~~tissue, cell type or organ~~; and

determining the presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cell-proliferative disease~~ based on comparing the total amount of free floating DNA and the fraction of free floating DNA that originates from the ~~tissue, cell type or organ of the test human~~, with that of a normal control value.

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3. (Previously presented) The method of any one of claims 1 and 2, wherein the sample is treated before the amount or presence of free floating DNA is determined.

4. (Previously presented) The method of claim 3, wherein the sample is treated by at least one centrifugation, filtering, heating, cooling, concentration and chemical treatment.

5. (Cancelled)

6. (Currently amended) The method of any one of claims 1 and 2, wherein the methylation pattern is characteristic for the particular ~~tissue, cell type or organ~~ and not found in other ~~tissues, cell types or organs~~ involved in the disease characterized by an increased amount of organ-specific free floating DNA ~~cell proliferative disease~~ of interest.

7. (Cancelled)

8. (Previously presented) The method of any one of claims 1 and 2, wherein the sample comprises at least one bodily fluid selected from the group consisting of whole blood, blood plasma, blood serum, urine, sputum, ejaculate, semen, tears, sweat, saliva, lymph fluid, bronchial lavage, pleural effusion, peritoneal fluid, meningeal fluid, amniotic fluid, glandular fluid, fine needle aspirates, nipple aspirate fluid, spinal fluid, conjunctival fluid, vaginal fluid, duodenal juice, pancreatic juice, bile and cerebrospinal fluid.

9. (Previously presented) The method of any one of claims 1 and 2, wherein determining the methylation pattern comprises subjecting the DNA to a chemical or enzymatic treatment that converts all unmethylated cytosines in the DNA into uracil but leaves position 5-methylated cytosines unmodified.

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10. (Currently amended) A method for detecting the presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cell-proliferative disease in a particular tissue, cell type or organ in a human individual~~, comprising:

obtaining a bodily fluid sample from a test human;

determining an amount or presence of free floating DNA that exhibits a DNA methylation pattern characteristic of a particular ~~tissue, cell type or organ~~;

determining whether there is an increased level of free floating DNA that originates from the ~~tissue, cell type or organ~~; and

determining a presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cell-proliferative disease associated with said tissue, cell type or organ~~, based on comparing the presence of such an increased level of free floating DNA that originates from the organ of the test human, with that of a normal control value.

11. (Currently amended) A method for detecting the presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cell-proliferative disease in a specific tissue, cell type or organ in a human individual~~, comprising:

obtaining a bodily fluid sample from a test human;

detecting an amount of total free floating DNA in the sample;

determining an amount of free floating DNA that originates from a specific ~~tissue, cell type or organ~~ by determining an amount of free floating DNA that exhibits a DNA methylation pattern characteristic of the a tissue, cell type or organ;

determining the fraction of total free floating DNA that originates from the specific ~~tissue, cell type or organ~~;

determining whether an increased level of free floating DNA that originates from the specific ~~tissue, cell type or organ~~ is present; and

determining the presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cell-proliferative disease associated with said tissue, cell type or organ~~, based on comparing the presence of such an increased level of free floating DNA that originates

from the organ of the test human, with that of a normal control value.

12. (Currently amended) A method for determining the fraction of total free floating DNA in a bodily fluid that originates from a specific ~~tissue, cell type or organ in a human individual~~, comprising:

obtaining a bodily fluid sample of a test human;

conditioning the sample to provide for binding of total free floating DNA to a surface;

binding an amount of the total free floating DNA to the surface;

detecting an amount of total free floating DNA by measuring the amount of DNA bound to the surface;

subjecting the surface comprising the bound DNA to at least one of a chemical and enzymatic treatment that converts all unmethylated cytosines in the DNA into uracil but leaves position-5 methylated cytosines unmodified;

amplifying the treated DNA;

analysing several methylation-specific positions in the treated DNA, and thereby determining an amount of DNA that exhibits an organ-specific ~~a tissue, cell type or organ-characteristic~~ DNA methylation pattern; and

comparing the amount of DNA that exhibits an organ-specific ~~a tissue, cell type or organ-characteristic~~ DNA methylation pattern to the amount of detected total free floating DNA, thereby determining the fraction of free floating DNA that originates from the specific ~~tissue, cell type or organ~~ in the total free floating DNA.

13. (Currently amended) The method of claim 12, further comprising:

determining whether an increased level of free floating DNA that originates from the specific ~~tissue, cell type or organ~~ is present; and

determining the presence of a disease characterized by an increased amount of organ-specific free floating DNA ~~cell-proliferative disease associated with said tissue, cell type or organ~~, based on comparing the presence of such an increased level of free floating DNA that originates

from the organ of the test human, with that of a normal control value.

14. (Previously presented) The method of any one of claims 1, 2, 10, 11, 12 and 13, wherein measuring the total amount of free floating DNA comprises use of at least one means selected from the group consisting of: intercalating fluorescent dyes or other dyes exhibiting changing fluorescence properties upon binding to DNA; hybridisation to DNA specific oligonucleotide or PNA oligomer probes; real time PCR assays; real time amplification procedures; UV-Vis absorbance; and amplification procedures with subsequent determination of the amount of product amplificate formed.

15. (Withdrawn) A kit for determining the total amount of free floating DNA in serum, comprising:

- a surface suitable to bind free floating DNA of a sample of bodily fluid;
- means for detecting an amount of DNA bound to the surface;
- reagents suitable to chemically or enzymatically modify the surface bound DNA to convert all unmethylated cytosines in the DNA into uracil but leave position-5 methylated cytosines unmodified;
- a container suitable to host the surface and said reagents; and
- means to control and adjust the temperature in the container.

16. (New) The method of any one of claims 1, 2, 10, 11, 12 and 13, wherein the disease characterized by an increased amount of organ-specific free floating DNA is cancer.